Neural Grafting in the Mammalian CNS
A. Björklund and U. Stenevi, editors

8

Preparation of Immature Central Nervous System Regions for Transplantation

ÅKE SEIGER

Department of Histology, Karolinska Institutet, Stockholm, Sweden.

Brain tissue undergoes dramatic structural changes during its development, from a tube of neuroepithelial cells to maturity when regions of gray matter, rich in neurons, and regions of white matter, rich in myelinated and unmyelinated nerve fibers, are intimately intertwined. Regions of adult central nervous system (CNS) dissected out from in situ brain have never been shown to survive transplantation. Therefore, for any transplantation procedure one is restricted to stages of immature donor brain tissue. There is a substantial increase in vulnerability to injuries with increasing age both for neurons and glia in CNS. Also, the demands for oxygen and nutrients increase dramatically with maturation. Taken together, the prerequisites for survival of CNS tissue upon grafting are unfavorable already at donor birth. The 'taking' of an immature piece of CNS upon transplantation is dependent on several factors, as follows.

(1) *Survival* of axotomized neurons in the graft upon mechanical dissection. This parameter influences different CNS regions in markedly different ways. Certain reticular neurons seem to tolerate extensive axotomies with good survival, whereas some other neurons with one axon die more frequently upon axotomy.

(2) *Proliferation.* The neurons and glial cells that survive the initial transplantation trauma will proliferate in relation to their individual developmental stage at grafting. The times of last mitotic divisions of neuroblasts in different CNS regions vary considerably. In the spinal cord and lower brain

stem mitotic activity in neurons ceases earlier than in cortical and subcortical forebrain areas. The degree of remaining neuronal mitotic activity in a region at the stage of grafting markedly influences the increase in size of the grafted brain regions. Proliferation of glial cells is considerably later than that of the principle neurons of most CNS areas, and will therefore be influenced by the degree of neuronal survival in a graft.

(3) *Placement.* Immature brain regions have been placed intracranially and in various extracranial positions such as in oculo, under the kidney capsule and in the testicle. This parameter interferes with the previous ones, and includes such variables as the speed by which the grafts are revascularized and possible trophic influences on the graft from the surrounding host, including the age of the recipient.

(4) *Technical,* including methods for dissecting the donor CNS as well as transplantation methods. Optimal donor stages for intraocular transplantation of a variety of fetal CNS regions are listed in Table I.

Staged pregnant rats are sacrificed and the two uterine horns dissected out as one piece. Fetal brain tissue survives well for 2–3 h when fetuses are left in the uterine horns at room temperature. Thus, there is ample time for dissection of fetal brain tissue pieces and no need to keep fetuses cold. In our experience, when grafting is performed within 2 h after killing the pregnant female, no adverse effects on taking have been noted. During this period, however, changes will be noted in the consistency of the fetal brain. Thus in the pallium it will be easier to remove the pia after a short while (5–10 min). The brain tissue itself will become increasingly soft, so that cuts will tend not to leave sharp edges and surfaces, again particularly in the pallium.

The embryonic brain is soft, with loosely aggregated neuroblasts, and it is thus easy to disrupt its organization by handling during the microdissections. A few pieces of advice on how to avoid such damage to the tissues to be grafted are as follows.

(a) When dissecting the brain out of the skull, never pinch, squeeze, or pull the brain tissue itself. Use the surrounding connective tissue as handles for the forceps.

(b) When cutting into the CNS itself, never cut more than once at each spot. Always cut *away* from the area to be grafted, and never touch it.

(c) If the cut surfaces of the isolated piece are ragged, trim away all small fragments protruding out of the smooth transplant surface.

(d) Move the grafts on the Petri dish as well as to the beaker in a fluid phase of sterile Ringer's solution by sucking the graft up in a Pasteur pipette. Thus, the CNS piece is not allowed to dry out, or to come into contact with the glassware unnecessarily.

(e) When occasionally the dissected brain piece shows signs of mechanical damage, or gets a shape or content other than expected, always discard the piece.

TABLE I

A GUIDE TO FETAL CNS TISSUE GRAFTING TO THE ANTERIOR EYE CHAMBER IN RATS

(From Olsen et al., 1983.)

	Optimal stage			
Region	Day	Crown-rump length	Expected growth (% volume increase)	Remarks
Parietal cerebral cortex	(15-) 16-18 (-22)	(15-) 18-24 (-45)	200-500	Transient blood sinusoids
Cerebellum	13-14 (-15)	11-13 (-15)	400-800	Myelinated parts conspicuous after maturation; by far most vulnerable to disturbances during grafting procedure
Hippocampus	(17-) 18-20 (-22)	(20-) 26-34 (-40)	200-600	
Dentate gyrus	(18-) 19-20 (-22)	(26-) 30-36 (-45)	300-600	
Entorhinal cortex	15-18	14-25	200-400	
Olfactory bulb	17-18 (-20)	19-25 (-35)	0-100	Easily fragmented at grafting
Caudate nucleus	(15-) 16 (-17)	(14-) 15-17 (-20)	200-300	Flat appearance on iris after maturation
Septal nuclei	(15-) 16-18 (-19)	(14-) 16-24 (-30)	0-100	
Tectum	18	23-26	0-100	
Substantia nigra region	(15-) 16 (-17)	(14-) 16-18 (-25)	100-200	
Dorsal raphe nucleus region	(16-) 17-18 (-20)	(16-) 18-25 (-35)	100-200	
Locus coeruleus region	(15-) 17-19 (-22)	(14-) 20-30 (-45)	50-200	
Spinal cord	(14-) 15-17 (-20)	(12-) 14-20 (-35)	100-400	

Parietal cerebral cortex is obtained as a longitudinal strip of the full depth of the cortex anlage (neopallium) from each side, further divided into 2–3 pieces approximately 1 × 1.5 mm each. The pia is peeled off from cortical anlage of 17-day and older fetuses, but can be left on pieces dissected from 16-day fetuses (Fig. 1.1, cf. Björklund et al., 1983a, b; Olson et al., 1979; Seiger and Olson, 1975).

Cerebellum. The cerebellar anlage is easily seen through the translucent skull of these young embryos. By unfolding the soft connective tissue over the developing IVth ventricle the thin rhombic lip is revealed. The medial two-thirds of the cerebellar anlage is cut out as a thin piece on each side (1–1.5 mm long and 0.5 mm wide). The lateral one-third of the anlage is excluded to avoid brain stem contamination (Fig. 1.2, cf. Hoffer et al., 1974).

Hippocampus and dentate gyrus. The hippocampal formation is reached through a paramedian sagittal cut in the cortex anlage. The dorsal portion of the hippocampal formation is cut out as one piece, approximately 3–5 mm long and 1–2 mm wide, to be further subdivided transversely in three to five 1-mm thick slices. From these slices the regio superior and inferior can be isolated as a 'hippocampal graft' from the 'dentate gyrus' piece, discarding the subicular region (Fig. 1.3, cf. Goldowitz et al., 1982, 1984a, b; Olson et al., 1977, 1979).

Caudate nucleus is reached from the unfolded cortex anlage (Fig. 1.1). Care must be taken to divide the thin caudate anlage from the adjacent tempero-parietal cortex tissue. A sagittal cut separates a caudate piece 3 mm rostro-caudally and 2 mm dorso-ventrally, retaining its normal rounded dorsal delineation. A further separation into two to three pieces is recommended before grafting (Fig. 1.1, cf. Olson et al., 1979).

Tectum is obtained as a paramedian piece of the developing superior colliculus approximately 2 mm wide and 1–1.5 mm in rostro-caudal direction all the way to the aqueduct, to be further divided in the midline into two similar pieces (Fig. 1.1, cf. Björklund et al., 1983a, b).

Substantia nigra region is reached from the ventral surface of the brain stem. A small median wedge approximately 2–3 mm wide and 1.5–2 mm long is cut out at the level of the vertex of the mesencephalic flexure. The wedge reaches dorsally halfway to the aqueduct. It is further divided sagittally in the midline to obtain unilateral ventro-medial tegmental pieces (Fig. 1.4, cf. Olson and Seiger, 1972; Olson et al., 1979; Seiger and Olson, 1977).

Dorsal raphe nucleus region. After a midsagittal cut through the tectum the aqueduct and the underlying tegmentum is revealed. The dorsal raphe nucleus is cut out as a median piece 2 mm wide and 1–1.5 mm long at the vertex of the mesencephalic flexure dorsally, just before the widening of the aqueduct into the developing IIIrd ventricle. This bilateral piece is then

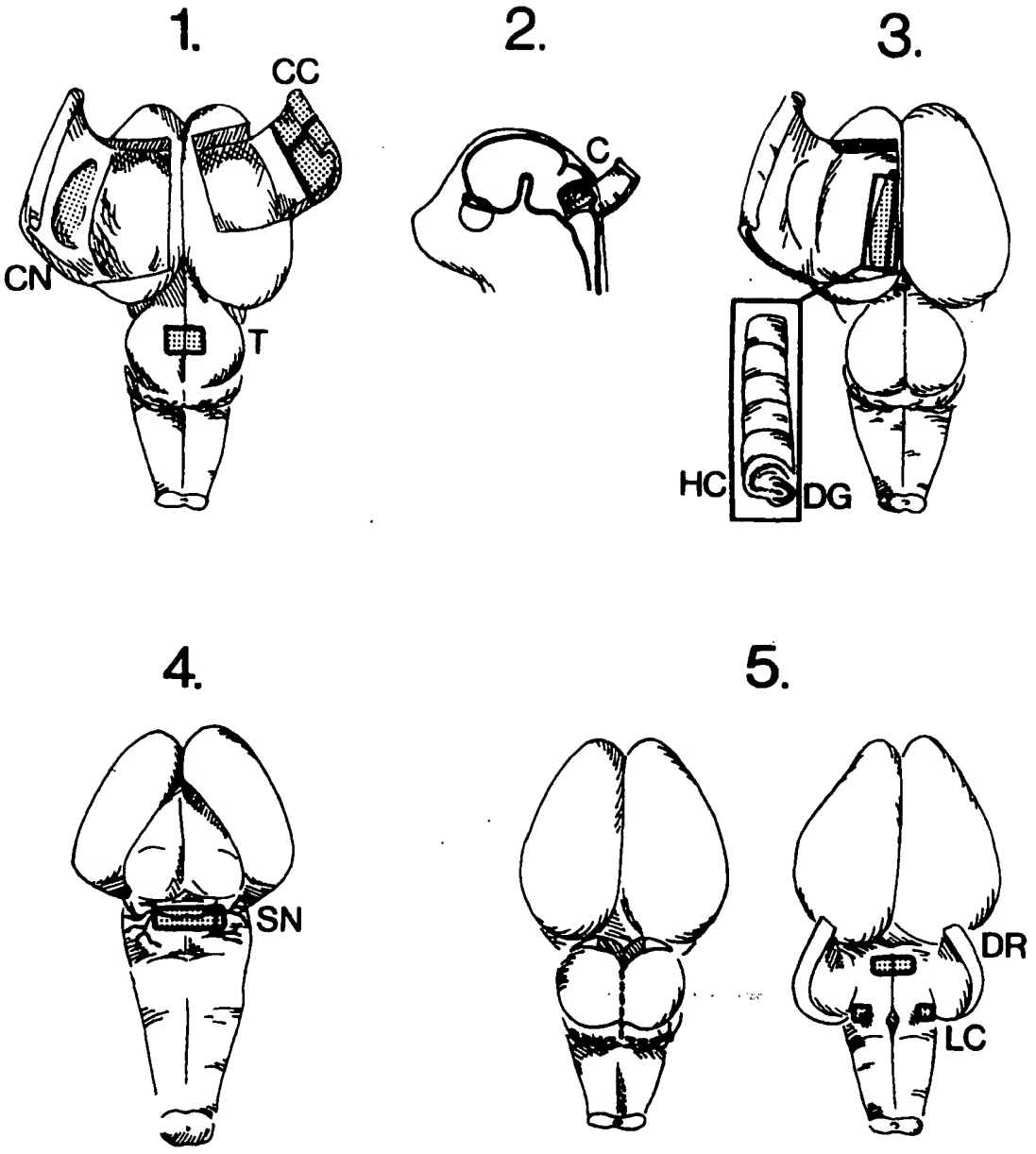

Figure 1. Schematic presentation of fetal CNS areas dissected for transplantation. All dissected areas are indicated by a dotted raster. For details see the text under each separate heading. Abbreviations: CC, cerebral cortex; CN, caudate nucleus; T, tectum; C, cerebellum; HC, hippocampus; DG, dentate gyrus; SN, substantia nigra region; DR, dorsal raphe nucleus region; LC, locus coeruleus region.

divided in the midline to give two similar pieces. Ventrally each piece should not exceed 1 mm in length, to avoid contamination of nigral neurons (Fig. 1.5, cf. Seiger and Olson, 1977).

Locus coeruleus region is most easily reached from the dorsal aspect of the brain. The developing tectum and cerebellum are cut in the midline leaving the floor of the IVth ventricle intact. Just caudal to the cerebellar anlage a transverse cut is made bilaterally in the roof of the ventricle all the way to its lateral edge, after which the cerebellum is unfolded. In the caudo-lateral part of the floor of the ventricle, just rostral to the pontine flexure, a small block of tissue is cut out, with the aim of removing as many of the locus coeruleus neuroblasts as possible, while minimizing the inclusion of other regions of the brain stem. In trying to get the same constituents of the piece to be transplanted regardless of the crown-rump length of the donor, larger pieces were taken from older donors than from younger ones. The size range was 1 × 1 × 1 mm to 2 × 1.5 × 1 mm (Fig. 1.5, cf. Björklund et al., 1983a, b; Goldowitz et al., 1984a, b; Olson and Seiger, 1972; Olson et al., 1979; Seiger and Olson, 1977).

Acknowledgements

Thanks are due to Dr. A.C. Granholm for making the schematic illustrations and to Mrs. Kristina Seiger for expert typing.

References

Björklund, H., Hoffer, B., Palmer, M., Seiger Å. and Olson, L. (1983a) Survival and growth of neurons with enkephalin-like immunoreactivity in fetal brain areas grafted to the anterior chamber of the eye, *Neuroscience*, 10: 1387–1398.

Björklund, H., Seiger, Å., Hoffer, B. and Olson, L. (1983b) Trophic effects of brain areas on the developing cerebral cortex. I. Growth and histological organization of intraocular grafts, *Develop. Brain Res.*, 6: 131–140.

Goldowitz, D., Seiger, Å. and Olson, L. (1982) Anatomy of the isolated area dentata grown in the rat anterior eye chamber, *J. comp. Neurol.*, 208: 382–400.

Goldowitz, D., Seiger, Å. and Olson, L. (1984a) Regulation of axonal ingrowth into area dentata as studied by sequential, double intraocular brain tissue transplantation, *J. comp. Neurol.*, in press.

Goldowitz, D., Seiger, Å. and Olson, L. (1984b) Degree of hyperinnervation of area dentata by locus coeruleus in the presence of septum or entorhinal cortex as studied by sequential intraocular triple transplantation, *Exp. Brain Res.*, in press.

Hoffer, B., Seiger, Å., Ljungberg, T. and Olson, L. (1974) Electrophysiological and cytological studies of brain homografts in the anterior chamber of the eye: maturation of cerebellar cortex in oculo, *Brain Res.*, 79: 165–184.

Olson, L. and Seiger, Å. (1972) Brain tissue transplanted to the anterior chamber of the eye: 1. Fluorescence histochemistry of immature catecholamine and 5-hydroxytryptamine neurons reinnervating the rat iris, *Z. Zellforsch.*, 135: 175–194.

Olson, L., Freedman, R., Seiger, Å. and Hoffer, B. (1977) Electrophysiology and cytology of hippocampal formation transplants in the anterior chamber of the eye. I. Intrinsic organization, *Brain Res.*, 119: 87–106.

Olson, L., Björklund, H., Hoffer, B., Palmer, M. and Seiger, Å. (1982) Spinal cord grafts: an intraocular approach to enigmas of nerve growth regulation, *Brain Res. Bull.*, 9: 519–537.

Olson, L., Seiger, Å., Hoffer, B. and Taylor, D. (1979) Isolated catecholaminergic projections from substantia nigra and locus coeruleus to caudate, hippocampus and cerebral cortex formed by intraocular sequential double brain grafts, *Exp. Brain Res.*, 35: 47–67.

Olson, L., Seiger, Å. and Strömberg, I. (1983) Intraocular transplantation in rodents. A detailed account of the procedure and examples of its use in neurobiology with special reference to brain tissue grafting. In S. Fedoroff (Ed.), *Advances in Cellular Neurobiology, Vol. 4*, Academic Press, New York, pp. 407–442.

Seiger, Å. and Olson, L. (1975) Brain tissue transplanted to the anterior chamber of the eye: 3. Substitution of lacking central noradrenaline input by host iris sympathetic fibers in the isolated cerebral cortex developed in oculo, *Cell Tiss. Res.*, 159: 325–338.

Seiger, Å. and Olson, L. (1977) Quantitation of fiber growth in transplanted central monoamine neurons, *Cell Tiss. Res.*, 179: 285–316.